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Antioxidant Action of the Antihypertensive Drug, Carvedilol, against Lipid Peroxidation

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ABSTRACT. The action of carvedilol, a vasodilating, β-adrenoceptor blocking agent, against lipid peroxidation has been the subject of many studies, but the results reported thus far are contradictory. In an attempt to define the antioxidant mechanism of carvedilol against lipid peroxidation, the dynamics of the action of carvedilol were studied in several oxidation systems. We investigated the reactivity of carvedilol toward radicals and its inhibitory effect on lipid peroxidation induced by several kinds of initiating species such as azo compounds and metal ions in solution, micelles, membranes, and low-density lipoprotein. Carvedilol exerted poor reactivity toward phenoxyl, alkoxyl, and peroxyl radicals in acetonitrile solution nor did it show an appreciable antioxidant effect against either the peroxyl radical-induced oxidation of methyl linoleate in acetonitrile or against phosphatidylcholine liposomal membranes in aqueous suspension. Carvedilol completely inhibited the ferric ion-induced oxidation of methyl linoleate micelles by sequestering ferric ions, but not by reducing hydroperoxide. It was shown that carvedilol enhanced the oxidation of micelles induced by either methemoglobin or peroxyl radical. Carvedilol, which was added exogenously, did not suppress the oxidation of isolated low-density lipoprotein induced by peroxyl radical or cupric ion. These results show that carvedilol does not act as a radical-scavenging antioxidant, but that it does act most efficiently as an antioxidant against ferric ion-induced oxidation by sequestering ferric ion. BIOCHEM PHARMACOL **59**;9:1069–1076, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. carvedilol; antioxidant; lipid peroxidation; radical; ferric ion; methemoglobin

Carvedilol, 1-[carbazole-(4)-oxy]-3-[(2-methoxy-phenoxy-ethyl)-amino]-2-propanol, is a vasodilator/β-adrenoceptor antagonist used for the treatment of hypertension [1, 2]. Carvedilol has been shown to have multiple functions such as neuroprotection [3–5] and myocardial [6, 7], and endothelial protection [8, 9]. Preventive effects of carvedilol against smooth muscle cell proliferation have been shown [10, 11]. It has also been reported that treatment with carvedilol both *in vitro* and *in vivo* increased the resistance of isolated LDL† against oxidation induced by macrophage or copper [12, 13]. In many of these studies, antioxidant properties of carvedilol are thought to account for the protective effects. The mechanisms of lipid peroxidation have been well documented [14, 15], and the action of antioxidants against this peroxidation has also been studied extensively [15]. The antioxidant properties of carvedilol have received much attention [4–9, 12, 13, 16–24]. However, the dynamics of the antioxidant action of carvedilol are not consistently understood yet. For example, it has been reported that carvedilol inhibits lipid peroxidation by scavenging free radicals [18], while another report concludes that carvedilol is not a radical scavenger but rather sequesters ferric ion [25]. In the present study, the dynamics of the action of carvedilol as an antioxidant against lipid peroxidation were studied in several oxidation systems to elucidate the molecular mechanisms of its antioxidant action.

MATERIALS AND METHODS

Materials

Carvedilol and probucol were supplied from Daiichi Pharmaceutical Co.. Troglitazone and 2*R*,4′*R*,8′*R*-α-tocopherol were supplied by Sankyo Co. and Eisai Co., respectively. Methyl linoleate obtained from Sigma was purified before use as described previously [26]. The hydrophilic azo compound AAPH and the lipophilic azo compounds AMVN [27] and MeO-AMVN [28], with the latter used as a peroxyl radical generator, were purchased from Wako Pure Chemical Industries and used as received. BHN, used as an alkoxyl radical generator, was prepared as previously re-

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† *Abbreviations:* LDL, low-density lipoprotein; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); MeO-AMVN, 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile); BHN, di-*tert*-butylhyponitrite; DPPD, *N*,*N*′-diphenyl-1,4-phenylenediamine; DPBQ, *N*,*N*′-diphenyl-1,4-benzoquinone diimine; BOOH, *tert*-butyl hydroperoxide; MeLOOH, methyl linoleate hydroperoxide; and PC, phosphatidylcholine.

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ported [29]. LDL was separated from human plasma of healthy donors and by ultracentrifugation within a density cut-off of 1.019 to 1.063 g/mL, followed by dialysis as described previously [30]. Galvinoxyl, DPPD, and other chemicals were of the highest grade available commercially.

Interaction of Antioxidant with Galvinoxyl

The interactions of carvedilol, probucol, troglitazone, or α-tocopherol with galvinoxyl were measured in acetonitrile with a spectrophotometer equipped with a rapid-mixing stopped-flow apparatus (RX-1000, Applied Photophysics) by following the decrease in maximum absorption of galvinoxyl at 429 nm [31].

Estimation of Reactivity of Antioxidant toward Alkoxyl and Peroxyl Radicals

The reactivities of carvedilol toward alkoxyl and peroxyl radicals were estimated from the competition with DPPD as follows. One molecule of DPPD reacts rapidly with two molecules of alkoxyl or peroxyl radical to give DPBQ, which has a strong absorption at 440 nm [28, 32]. Appropriate amounts of DPPD and either BHN or AMVN were incubated in acetonitrile at 37° under air in the absence or presence of antioxidant, and the formation of DPBQ was followed spectrophotometrically at 440 nm. The reactivity of the antioxidant toward alkoxyl and peroxyl radicals was assessed from the extent of reduction of formation of DPBQ by the test antioxidant.

Inhibition of Oxidation of Lipids by Carvedilol

The oxidations of methyl linoleate and soybean PC liposomes were carried out at 37° in air. Methyl linoleate was incubated in acetonitrile in the presence of AMVN and an appropriate amount of antioxidant, and the formation of methyl linoleate hydroperoxides was measured by HPLC equipped with a reversed-phase LC-18 column (particle size 5 μm; 4.6 mm × 25 cm; Supelco). Methanol: H_2O (95:5, v/v) was used as an eluent at a flow rate of 1.0 mL/min, and the hydroperoxides were detected by UV absorption at 234 nm [33]. The multilamellar PC liposomal membranes were prepared as reported previously [34]. PC, AMVN, and antioxidants, when used, were incorporated into the membranes simultaneously by dissolving them into the solvent before preparation of the membranes. The PC hydroperoxides were analyzed by absorption at 234 nm by HPLC using an LC-Si column (particle size 5 μm; 4.6 mm × 25 cm; Supelco), and methanol: 40 mM phosphate buffer (90:10 v/v) was used as an eluent at a flow rate of 1.0 mL/min.

The oxidation of methyl linoleate was performed in aqueous dispersions at 37° in air. The methyl linoleate micelles were prepared as reported previously [35] by using SDS as a surfactant. Carvedilol was added as a methanol solution at the time of preparation of micelles or at an appropriate time during oxidation. The oxidation was induced by adding BHN or AMVN to aqueous dispersion as a methanol solution. AAPH, $CuCl_2$, $FeCl_3$, or methemoglobin was added as an aqueous solution. Hydrogen peroxide, BOOH, or MeLOOH was added as a methanol solution. The rate of oxidation was followed by measuring oxygen uptake with an oxygen monitor equipped with a Clark-type oxygen electrode (YSI Model 5300).

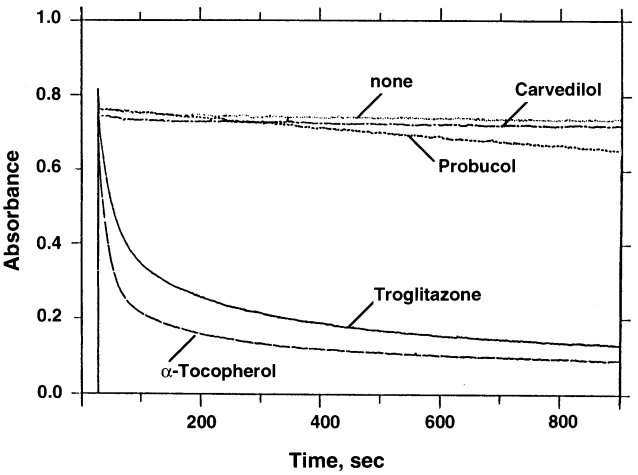

FIG. 1. Reaction of carvedilol with galvinoxyl. Antioxidant (10 μM) and galvinoxyl (5 μM) in acetonitrile were reacted, and the consumption of galvinoxyl was followed by its absorption at 429 nm. The data for α-tocopherol, troglitazone, and probucol are also included for comparison.

Oxidation of LDL

The oxidation of LDL (0.1 mg protein/mL PBS) was induced by either $CuCl_2$ or MeO-AMVN. Carvedilol was added as a methanol solution prior to the induction of the oxidation. The oxidation was followed by measuring the increase in conjugated diene formation with a spectrophotometer at 234 nm [36].

RESULTS

Reactivity of Carvedilol toward Radical

As mentioned above, some reports indicate radical-scavenging activity of carvedilol, whereas other reports deny it. Hence, the reactivities of carvedilol toward radicals were first measured. Galvinoxyl radical has often been used in the estimation of reactivity of antioxidant toward radicals. Potent radical-scavenging antioxidants having reactive hydrogens react rapidly with galvinoxyl. It was found that while α-tocopherol and troglitazone very rapidly reduced the absorption of galvinoxyl radical at 429 nm, carvedilol did not reduce it at an appreciable rate (Fig. 1). The capability of carvedilol to act as a hydrogen donor is even smaller than that of probucol [33]. Secondly, the reactivities toward alkoxyl and peroxyl radicals were measured. The actual rate of free radical flux was measured by using DPPD as a radical scavenger. One molecule of DPPD scavenges two radicals rapidly to give DPBQ [28, 32], which has a

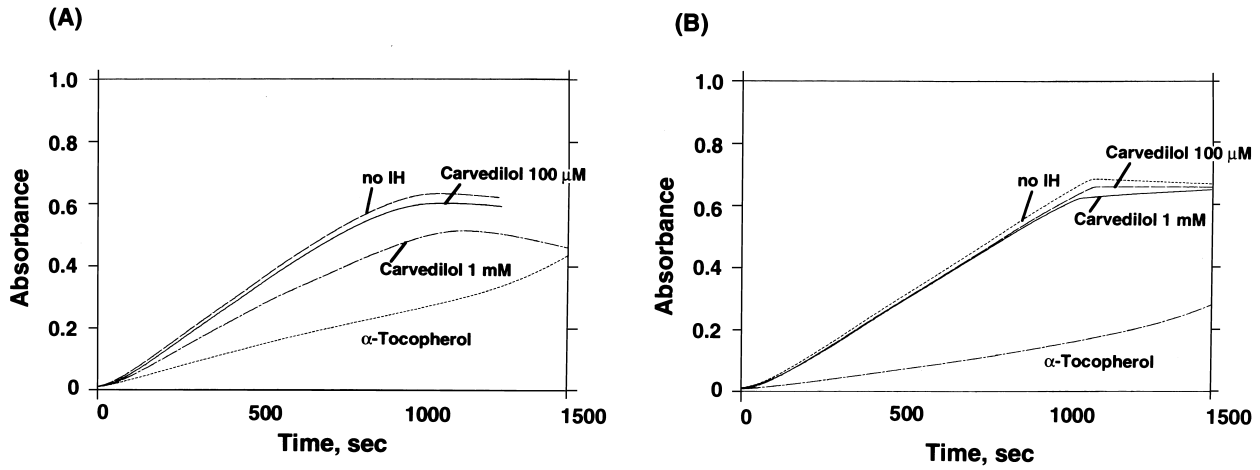

FIG. 2. Reactivity of carvedilol toward alkoxyl radical and peroxyl radical. DPPD (100 μM) was incubated with either (A) BHN (20 mM) or (B) AMVN (40 mM) at 37° in the absence (no antioxidant [IH]) and presence of antioxidant in acetonitrile, and the formation of DPBQ was followed at 440 nm. While a radical-scavenging antioxidant such as α-tocopherol (100 μM) suppressed the formation of DPBQ, carvedilol showed little effect.

strong absorption at 440 nm. This absorption increased with time due to the formation of DPBQ from DPPD via the reaction with the radicals generated from BHN or AMVN. In Fig. 2 is shown the increase in absorbance at 440 nm observed when DPPD and BHN or AMVN were incubated at 37° in air. These radical initiators induced the increase in the absorption at 440 nm linearly with time in the absence of antioxidant, the absorbance became constant when all the DPPD had been converted to DPBQ. The plateau absorbance was directly proportional to the initial DPPD concentration. The addition of α-tocopherol to this mixture suppressed the formation of DPBQ, apparently because α-tocopherol competed with DPPD in scavenging alkoxyl or peroxyl radicals. On the other hand, carvedilol exerted little effect on the formation of DPBQ, suggesting that carvedilol was not capable of competing with DPPD in scavenging these radicals.

Antioxidant Action of Carvedilol against Lipid Peroxidation in Solution and Liposomal Membranes

The antioxidant activity of carvedilol was assessed in the oxidation of methyl linoleate induced by AMVN in acetonitrile solution. The rate of spontaneous oxidation of methyl linoleate in acetonitrile at 37° under air was quite small (data not shown), but the addition of AMVN induced the oxidation and the accumulation of methyl linoleate hydroperoxide was observed at a constant rate without any appreciable induction period (Fig. 3A). A small amount of troglitazone suppressed the oxidation and produced a clear induction period, after which oxidation proceeded at a similar rate as oxidation without antioxidant. Similar results have been observed for α-tocopherol. On the other hand, the antioxidant activity of carvedilol was extremely small. Liposomal membranes are often used as a model of biological membranes. The antioxidant action of carvedilol was studied in the oxidations of soybean PC liposomal membranes. Antioxidant and AMVN were incorporated into multilamellar liposomal membranes simultaneously. The results summarized in Fig. 3B show that α-tocopherol efficiently suppressed the oxidation of PC membranes and produced a clear induction period. The rates of oxidation after the induction period were the same as that in the absence of antioxidant. Carvedilol acted as a weak antioxidant and reduced the rate of oxidation at the high concentration.

Antioxidant Activity of Carvedilol in Oxidation of Methyl Linoleate Micelles

The antioxidant action of carvedilol against the oxidations of methyl linoleate micelles in aqueous dispersions, which were induced by several initiating species, was studied. The extent of oxidation was followed by measuring oxygen consumption. It was found that carvedilol did not inhibit the oxidation induced by alkoxyl radical (data not shown) and even enhanced that induced by peroxyl radical (Fig. 4). It was found that an equimolar amount of carvedilol completely inhibited the oxidation of micelles induced by ferric chloride. The complete inhibition of ferric ion-induced oxidation by carvedilol was observed under the condition in which the ratio of carvedilol to ferric ion was higher than 1 (Fig. 5A). Addition of an excess amount of either MeLOOH or BOOH did not resume the oxidation (Fig. 5B).

The antioxidant activity of carvedilol was assessed in the oxidation of micelles induced by methemoglobin. Although methemoglobin contains ferric ion in the molecule, carvedilol did not inhibit methemoglobin-induced oxidation of micelles, but rather enhanced it significantly (Fig. 6). The enhancement of the reaction of methemoglobin-induced oxidation by carvedilol in micelles was confirmed by following a change in the spectrum of methemoglobin in the absence and presence of carvedilol. Methemoglobin exhibited absorption at 400 nm in methyl linoleate micelle

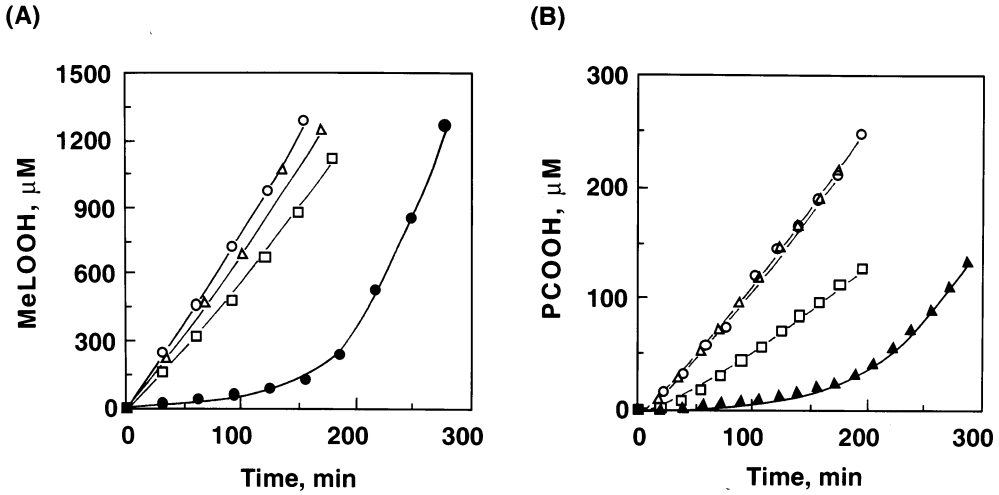

FIG. 3. Effect of carvedilol on lipid peroxidation in solution and membranes induced by peroxyl radical. (A) Methyl linoleate (453 mM) was oxidized in acetonitrile in the presence of AMVN (0.20 mM) without antioxidant (○), with 20 (△) or 200 μM (□) carvedilol, or with 5 μM troglitazone (●) at 37° in air, and MeLOOHs were measured as described in Materials and Methods. (B) Soybean PC (5.15 mM) multilamellar liposomal membranes containing AMVN (0.50 mM) without antioxidant (○), with 20 (△) or 200 μM (□) carvedilol, or with 2 μM α-tocopherol (▲) were incubated at 37° in air, and the formation of PC hydroperoxides (PCOOH) was followed by HPLC as described in Materials and Methods.

dispersion, with the absorbance decreasing with time (Fig. 7A). Carvedilol showed no absorption at about 400 nm and enhanced the change in the absorbance of methemoglobin (Fig. 7B).

Inhibition of Oxidation of LDL by Carvedilol

The antioxidant activity of carvedilol in the oxidation of LDL is of great interest. The oxidation of human LDL was carried out by using either cupric ion or a lipophilic radical initiator, MeO-AMVN, and the extent of the oxidation was measured by following the increase in absorbance at 234 nm. As expected, carvedilol did not show an inhibitory effect on the peroxyl radical-induced oxidation of LDL (data not shown). It was also shown that carvedilol did not reduce the rate of formation of conjugated diene in the copper-induced oxidation of LDL to an appreciable degree (Fig. 8).

DISCUSSION

The antioxidant exerts its function by different mechanisms such as radical scavenging, peroxide reduction, and metal ion sequestration [37]. Peroxyl radical is one of the

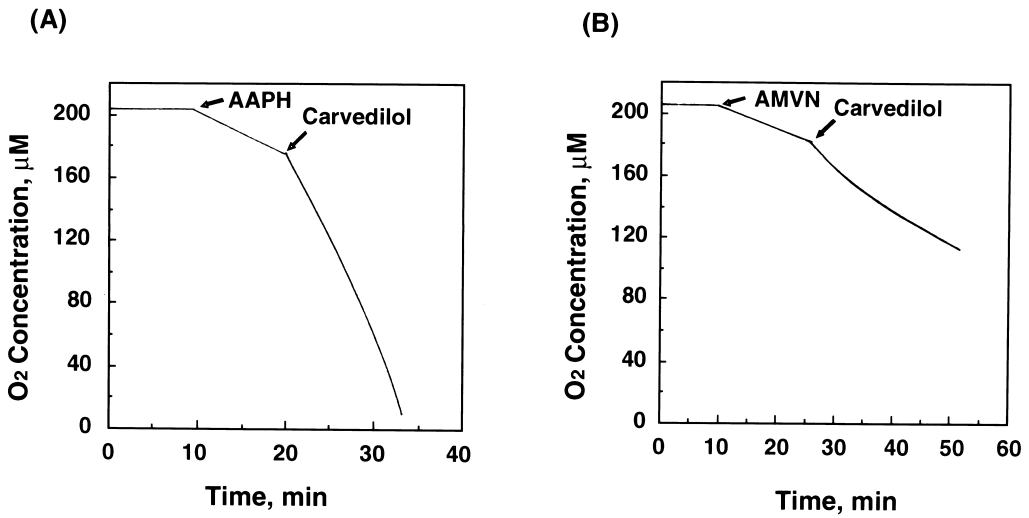

FIG. 4. Effect of carvedilol on peroxyl radical-induced oxidation of methyl linoleate micelles. Oxidation of methyl linoleate micelles (75 mM/0.5 M SDS in H_2O) was induced by either (A) a hydrophilic radical initiator, AAPH (2 mM), or (B) a lipophilic radical initiator, AMVN (2 mM), at 37°. The oxidation was followed by oxygen consumption, and carvedilol (100 μM) was added as a methanol solution at the point indicated in the figure.

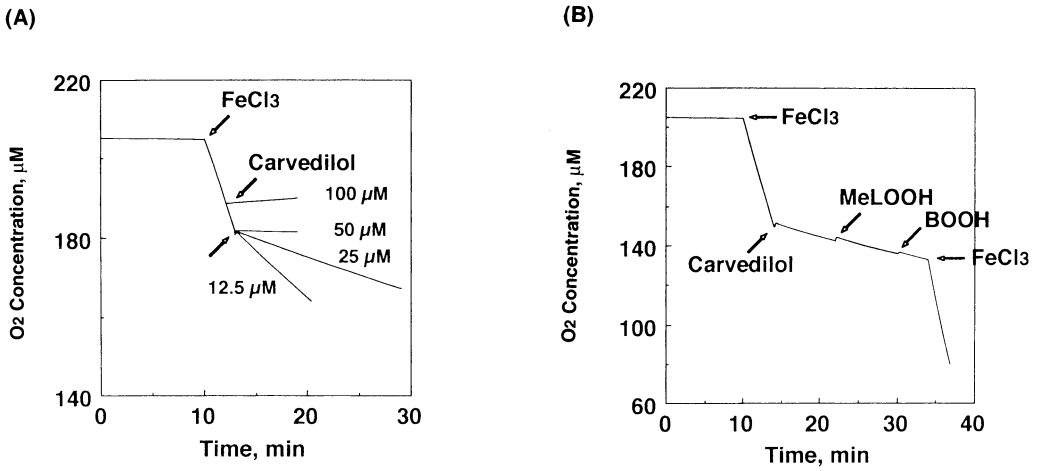

FIG. 5. Inhibition of ferric ion-induced oxidation of methyl linoleate micelles by carvedilol. (A) Oxidation of methyl linoleate micelles (75 mM/0.5 M SDS in H_2O) was induced by 50 μM $FeCl_3$ and inhibited by equimolar carvedilol. (B) Oxidation of methyl linoleate micelles (75 mM/0.5 M SDS in H_2O) was induced by 100 μM $FeCl_3$ and inhibited by equimolar carvedilol. The oxidation was not restarted by the addition of MeLOOH (100 μM) or BOOH (300 μM).

most important radicals to act as a chain-carrying species in lipid peroxidation. Antioxidants such as α-tocopherol and ubiquinol, which scavenge peroxyl radical very rapidly, act as chain-breaking antioxidants. We first investigated the reactivity of carvedilol toward radicals. It was shown clearly in Figs. 1 and 2 that carvedilol does not act as a potent radical scavenger. A carbazole group of carvedilol has been attributed to its antioxidant properties. It has previously been shown [38, 39] that carbazoles with a free hydroxyl group suppress oxidation effectively but those without do not. Since the carbazole group of carvedilol does not have a free hydroxyl group, poor activity of carvedilol as a radical scavenger seems reasonable. On the other hand, Yue *et al.* [18] reported that carvedilol acted as a radical-scavenging antioxidant, which is not consistent with our results. However, they used iron as an initiating species, and their results can also be explained by sequestration of iron by carvedilol.

Secondly, the hydroperoxide-reducing activity of carvedilol was studied. The oxidation of methyl linoleate micelles induced by ferric ion is dependent on MeLOOH, which is contained in the micelles [35, 40]. It was previously shown that ebselen, which mimics glutathione peroxidase, completely inhibited metal-induced lipid peroxidation by reducing seeding hydroperoxide [40–43]. If carvedilol inhibits ferric ion-induced oxidation of micelles by reducing hydroperoxide, the addition of an excess amount of hydroperoxide should resume oxidation. Since, as shown in Fig. 5B, the addition of a high concentration of MeLOOH and BOOH showed no effect, the reducing activity of hydroperoxide by carvedilol is ruled out. Indeed, we confirmed that cholesteryl ester hydroperoxide was not

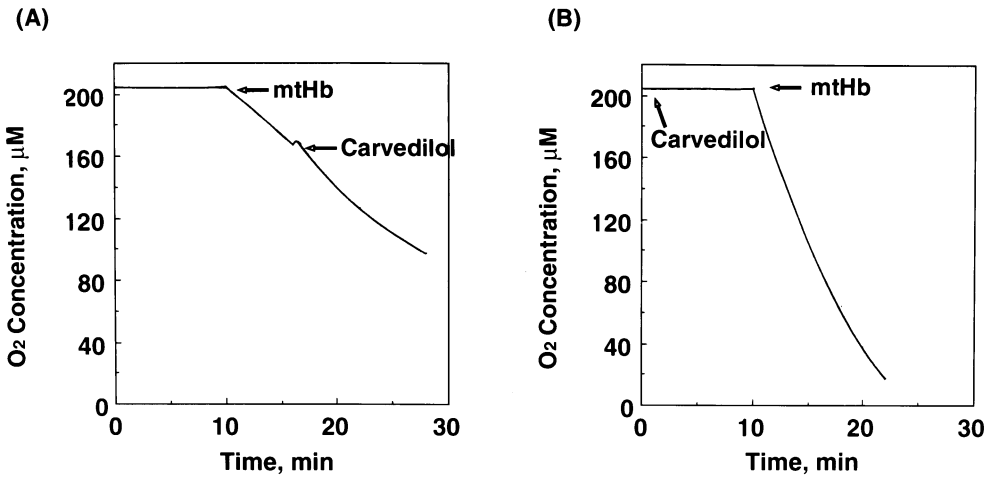

FIG. 6. Effect of carvedilol on oxidation of methyl linoleate micelles induced by methemoglobin. (A) Oxidation of methyl linoleate micelles (15 mM/0.5 M SDS in H_2O) was induced by 1 μM methemoglobin (mtHb). The addition of carvedilol (100 μM) after onset of the oxidation showed no effect. (B) When methyl linoleate micelles (15 mM/0.5 M SDS in H_2O) containing 100 μM carvedilol were oxidized by 1 μM mtHb, the rate of oxidation was greater than that without carvedilol.

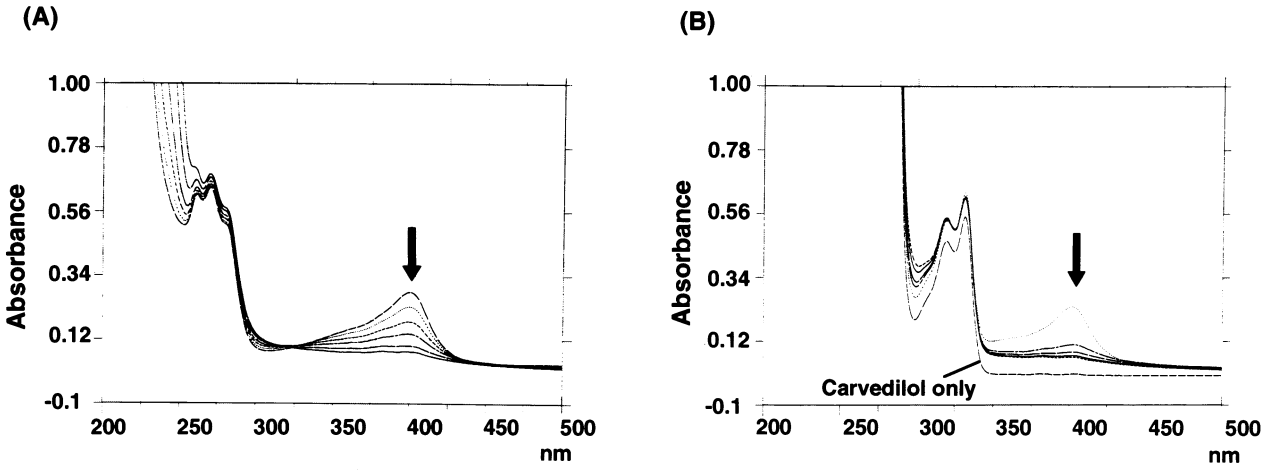

FIG. 7. Enhancement by carvedilol of the change in spectrum of methemoglobin in the oxidation of micelles. Methemoglobin (1 μM) was incubated with methyl linoleate micelles (15 mM/0.5 M SDS in H_2O) in the absence (A) and presence of 100 μM carvedilol (B) at 37°. The spectrum was measured every 3 min.

reduced by carvedilol. On the other hand, carvedilol inhibited the ferric ion-induced oxidation of micelles in a concentration-dependent manner. The equimolar carvedilol to ferric ion showed a complete inhibitory effect. The addition of an excess amount of ferric ion to the oxidation system in which carvedilol stopped oxidation restarted this oxidation. These results are in agreement with those of Tadolini and Franconi [25], who showed that carvedilol could make a complex with ferric ion in a 1:1 ratio in methanol/H_2O solution. The present study showed that carvedilol, a lipophilic compound which is located in the lipid phase, could react with ferric ion added to the aqueous phase. The rate of oxidation of micelles induced by a certain concentration of ferric ion was the same as that induced by the same concentration of ferric ion which remained after sequestration by carvedilol in a 1:1 ratio (data not shown).

We investigated the action of carvedilol against lipid peroxidation induced by methemoglobin, which may be important *in vivo*. Although methemoglobin contains ferric ion, contrary to our expectations, carvedilol did not inhibit the methemoglobin-induced oxidation of methyl linoleate micelles but even enhanced it. The reason for this is not clear at present. Further investigation of the several different oxidation systems is required.

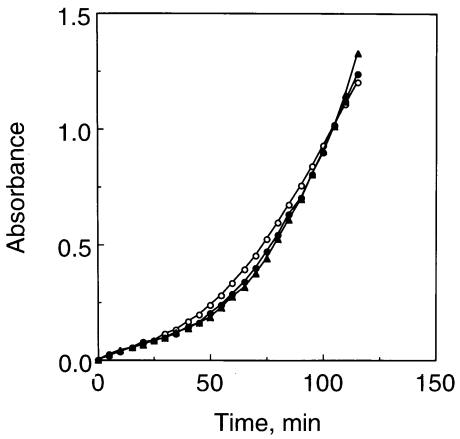

FIG. 8. Effect of carvedilol on oxidation of LDL induced by copper. LDL (0.1 mg protein/mL PBS) was oxidized by 2 μM $CuCl_2$ in the absence (○) or presence of 5 (●) and 10 μM (▲) carvedilol at 37° in air. The oxidation was followed by measuring the increase in absorbance at 234 nm.

Finally, we studied the antioxidant potency of carvedilol against LDL oxidation induced by either a radical initiator or copper ion. As observed in other oxidation systems, carvedilol did not inhibit the peroxyl radical-induced oxidation of LDL. The antioxidant effect of carvedilol on the copper-induced oxidation of LDL has been studied both *in vivo* and *in vitro* [12, 13]. In the present study, it was shown that carvedilol did not suppress the oxidation of LDL induced by copper to an appreciable degree. We also confirmed that carvedilol did not inhibit the copper-induced oxidation of methyl linoleate micelles (data not shown). These results are not in agreement with those of Yue *et al.* [12], who observed antioxidant activity of carvedilol against LDL oxidation induced by macrophage and copper. It is known that metal is required for oxidation by macrophage, and it may be possible that carvedilol suppressed LDL oxidation induced by macrophage by sequestering iron. The effect of carvedilol on copper-induced LDL oxidation was reported to be less significant than that for macrophage-induced oxidation [12]. Furthermore, the thiobarbituric acid assay used in their work is affected by metal ion and chelating agents. It was reported that treatment with carvedilol *in vivo* increased the lag time in copper-induced LDL oxidation [13]. Considering the concentration of carvedilol in plasma of patients treated with carvedilol [44], it is difficult to explain the antioxidant effect in LDL particle by carvedilol alone, but its metabolite, having a hydroxyl group on the phenyl ring, might act as an antioxidant against LDL oxidation [45].

It may be concluded that carvedilol does not act as a

radical-scavenging antioxidant and does not reduce hydroperoxides, but that it does act as an antioxidant against iron-induced lipid peroxidation by sequestering ferric ion.

References

1. Feuerstein GZ and Ruffolo RR Jr, Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. *Eur Heart J* **16** (Suppl F): 38–42, 1995.
2. Dunn CJ, Lea AP and Wagstaff AJ, Carvedilol. A reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. *Drugs* **54:** 161–185, 1997.
3. Lysko PG, Lysko KA, Webb CL and Feuerstein GZ, Neuroprotective effects of carvedilol, a new antihypertensive, at the *N*-methyl-D-aspartate receptor. *Neurosci Lett* **148:** 34–38, 1992.
4. Lysko PG, Lysko KA, Yue TL, Webb CL, Gu JL and Feuerstein GZ, Neuroprotective effects of carvedilol, a new antihypertensive agent, in cultured rat cerebellar neurons and in gerbil global brain ischemia. *Stroke* **23:** 1630–1635, 1992.
5. Yue TL, Lysko PG, Brone FC, Gu JL, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new antihypertensive drug with unique antioxidant activity: Potential role in cerebroprotection. *Ann N Y Acad Sci* **738:** 230–242, 1994.
6. Feuerstein GZ, Yue TL, Cheng HY and Ruffolo RR Jr, Myocardial protection by the novel vasodilating beta-blocker, carvedilol: Potential relevance of anti-oxidant activity. *J Hypertens* **11** (Suppl 1): S41–S48, 1993.
7. Sugawara H, Tobise K and Kikuchi K, Antioxidant effects of calcium antagonists on rat myocardial membrane lipid peroxidation. *Hypertens Res* **19:** 223–228, 1996.
8. Yue TL, McKenna PJ, Gu JL, Cheng HY, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new antihypertensive agent, prevents lipid peroxidation and oxidative injury to endothelial cells. *Hypertension* **22:** 922–928, 1993.
9. Yue TL, McKenna PJ, Gu JL, Cheng HY, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new vasodilating β adrenoceptor blocker antihypertensive drug, protects endothelial cells from damage initiated by xanthine–xanthine oxidase and neutrophils. *Cardiovasc Res* **28:** 400–406, 1994.
10. Ohlstein EH, Douglas SA, Sung CP, Yue TL, Louden C, Arleth A, Poste G, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a cardiovascular drug, prevents vascular smooth muscle cell proliferation, migration, and neointimal formation following vascular injury. *Proc Natl Acad Sci USA* **90:** 6189–6193, 1993.
11. Sung CP, Arleth AJ and Ohlstein EH, Carvedilol inhibits vascular smooth muscle cell proliferation. *J Cardiovasc Pharmacol* **21:** 221–227, 1993.
12. Yue TL, McKenna PJ, Lysko PG, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new antihypertensive, prevents oxidation of human low density lipoprotein by macrophages and copper. *Atherosclerosis* **97:** 209–216, 1992.
13. Maggi E, Marchesi E, Covini D, Negro C, Perani G and Bellomi G, Protective effects of carvedilol, a vasodilating β-adrenoceptor blocker, against *in vivo* low density lipoprotein oxidation in essential hypertension. *J Cardiovasc Pharmacol* **27:** 532–538, 1996.
14. Porter NA, Caldwell SE and Mills KA, Mechanisms of free radical oxidation of unsaturated lipids. *Lipids* **30:** 277–290, 1995.
15. Noguchi N and Niki E, Chemistry of active oxygen species and antioxidants, In: *Antioxidant Status, Diet, Nutrition, and Health* (Ed. Papas AM), pp. 3–20. CRC Press, Boca Raton, 1999.
16. Feuerstein GZ and Ruffolo RR Jr, Carvedilol, a novel vasodilating β-blocker with the potential for cardiovascular organ protection. *Eur Heart J* **17** (Suppl B): 24–29, 1996.
17. Yue TL, McKenna PJ, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new β-adrenoceptor antagonist and vasodilator antihypertensive drug, inhibits superoxide release from human neutrophils. *Eur J Pharmacol* **214:** 277–280, 1992.
18. Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, Lysko KA, Davis LL and Feuerstein GZ, Carvedilol, a new vasodilator and β-adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J Pharmacol Exp Ther* **263:** 92–98, 1992.
19. Christopher TA, Lopez BL, Yue TL, Feuerstein GZ, Ruffolo RR Jr and Ma X, Carvedilol, a new β-adrenoceptor blocker, vasodilator and free-radical scavenger, exerts an anti-shock and endothelial protective effect in rat splanchnic ischemia and reperfusion. *J Pharmacol Exp Ther* **273:** 64–71, 1995.
20. Lopez BL, Christopher TA, Yue TL, Ruffolo RR Jr, Feuerstein GZ and Ma XL, Carvedilol, a new β-adrenoceptor blocker antihypertensive drug, protects against free-radical-induced endothelial dysfunction. *Pharmacology* **51:** 165–173, 1995.
21. Kramer JH and Weglicki WB, A hydroxylated analog of the β-adrenoceptor antagonist, carvedilol, affords exceptional antioxidant protection to postischemic rat hearts. *Free Radic Biol Med* **21:** 813–825, 1996.
22. Ma XL, Yue TL, Lopez BL, Barone FC, Christopher TA, Ruffolo RR Jr and Feuerstein GZ, Carvedilol, a new β-adrenoceptor blocker and free radical scavenger, attenuates myocardial ischemia–reperfusion injury in hypercholesterolemic rabbits. *J Pharmacol Exp Ther* **277:** 128–136, 1996.
23. Ruffolo RR Jr and Feuerstein GZ, Pharmacology of carvedilol: Rationale for use in hypertension, coronary artery disease, and congestive heart failure. *Cardiovasc Drugs Ther* **11** (Suppl 1): 247–256, 1997.
24. Rabasseda X, Carvedilol: An effective antihypertensive drug with antiischemic/antioxidant cardioprotective properties. *Drugs Today* **34:** 905–926, 1998.
25. Tadolini B and Franconi F, Carvedilol inhibition of lipid peroxidation. A new antioxidative mechanism. *Free Radic Res* **29:** 377–387, 1998.
26. Komuro E, Takahashi M, Morita T, Tsuchiya J, Arakawa Y, Yamamoto Y, Niki E, Sugioka K and Nakano M, Inhibition of peroxidations of lipids and membranes by estrogens. *J Phys Org Chem* **3:** 309–315, 1990.
27. Niki E, Free radical initiators as source of water- or lipid-soluble peroxyl radicals. In: *Methods in Enzymology* (Eds. Packer L and Glazer AN), Vol. 186, pp. 100–108. Academic Press, New York, 1990.
28. Noguchi N, Yamashita H, Gotoh N, Yamamoto Y, Numano R and Niki E, 2,2′-Azobis (4-methoxy-2,4-dimethylvaleronitrile), a new lipid-soluble azo initiator: Application to oxidations of lipids and low-density lipoprotein in solution and in aqueous dispersions. *Free Radic Biol Med* **24:** 259–268, 1998.
29. Kigoshi M, Sato K and Niki E, Oxidations of lipids induced by dioctadecyl hyponitrite and di-*t*-butyl hyponitrite in organic solution and aqueous dispersions. Effect of reaction medium and size of radicals on efficiency of chain initiation. *Bull Chem Soc Jpn* **66:** 2954–2959, 1993.
30. Ramos P, Gieseg SP, Schuster B and Esterbauer H, Effect of temperature and phase transition on oxidation resistance of low density lipoprotein. *J Lipid Res* **36:** 2113–2128, 1995.
31. Iwatsuki M, Komuro E and Niki E, Antioxidant activities of aminophenols against oxidation of methyl linoleate in solution. *Bull Chem Soc Jpn* **68:** 620–624, 1995.
32. Takahashi M, Tsuchiya J and Niki E, Oxidation of lipids. XVI. Inhibition of autoxidation of methyl linoleate by diarylamines. *Bull Chem Soc Jpn* **62:** 1880–1884, 1989.
33. Gotoh N, Shimizu K, Komuro E, Tsuchiya J, Noguchi N and

Niki E, Antioxidant activities of probucol against lipid peroxidations. *Biochim Biophys Acta* **1128:** 147–154, 1992.
34. Yamamoto Y, Niki E, Kamiya Y and Shimasaki H, Oxidation of lipids. VII. Oxidation of phosphatidylcholines in homogeneous solution and in water dispersion. *Biochim Biophys Acta* **795:** 332–340, 1984.
35. Yoshida Y and Niki E, Oxidation of methyl linoleate in aqueous dispersions induced by copper and iron. *Arch Biochem Biophys* **295:** 107–114, 1992.
36. Esterbauer H, Striegel G, Puhl H and Rotheneder M, Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic Res Commun* **6:** 67–75, 1989.
37. Niki E, Noguchi N, Tsuchihashi H and Gotoh N, Interaction among vitamin C, vitamin E, and β-carotene. *Am J Clin Nutr* **62** (Suppl 6): 1322S–1326S, 1995.
38. Yamamoto Y, Yamamoto Y, Niki E, Gee CA and Willson RL, Antioxidant activity of 6-hydroxy-1,4-dimethylcarbazole. *J Jpn Oil Chem Soc* **41:** 622–628, 1992.
39. Iwatsuki M, Niki E and Kato S, Antioxidant activities of natural and synthetic carbazoles. *Biofactors* **4:** 123–128, 1993.
40. Noguchi N, Yoshida Y, Kaneda H, Yamamoto Y and Niki E, Action of ebselen as an antioxidant against lipid peroxidation. *Biochem Pharmacol* **44:** 39–44, 1992.
41. Maiorino M, Roveri A and Ursini F, Antioxidant effect of Ebselen (PZ 51): Peroxidase mimetic activity on phospholipid and cholesterol hydroperoxides vs free radical scavenger activity. *Arch Biochem Biophys* **295:** 404–409, 1992.
42. O'Leary VJ, Darley-Usmar VM, Russell LJ and Stone D, Pro-oxidant effects of lipoxygenase-derived peroxides on copper-initiated oxidation of low-density lipoprotein. *Biochem J* **282:** 631–634, 1992.
43. Noguchi N, Gotoh N and Niki E, Effects of ebselen and probucol on oxidative modifications of lipid and protein of low density lipoprotein induced by free radicals. *Biochim Biophys Acta* **1213:** 176–182, 1994.
44. Saijonmaa O, Metsarinne K and Fyhrquist F, Carvedilol and its metabolites suppress endothelin-1 production in human endothelial cell culture. *Blood Press* **6:** 24–28, 1997.
45. Yue TL, McKenna PJ, Lysko PG, Gu JL, Lysko KA, Ruffolo RR Jr and Feuerstein GZ, SB 211475, a metabolite of carvedilol, a novel antihypertensive agent, is a potent antioxidant. *Eur J Pharmacol* **251:** 237–243, 1994.